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The Effects of Hydroxyethyl Starch Compared with Gelofusine on Activated Endothelium and the Systemic Inflammatory Response Following Aortic Aneurysm Repair

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Objective. *To investigate the effect of HES, used as a plasma volume expander, on endothelial cell activation induced by ischaemia-reperfusion in humans.*
Material and Methods. *Forty patients undergoing elective infrarenal aneurysm repair were randomised to receive either gelatine or hydroxyethyl starch solution as plasma expanders. The anaesthetic technique was standardised. All patients received the same crystalloid as per standard protocol. Urine samples and blood samples were collected at various times for assessment of microalbuminuria and von Willebrand factor (vWf) and CRP.*
Results. *The peak C-reactive protein was significantly lower in the patients treated with HES than those treated with gelofusine [142 mg/L (113,196 mg/L) vs 246 mg/L (189,291 mg/L) mg/L, $P<0.01$, Mann-Whitney test]. The peak ACR was also significantly lower in the HES treated patients (9.3 mg/mmol vs 23.3 mg/mmol, $P<0.05$). The plasma level of vWf was significantly higher in the gelofusine treated patients than those treated with HES [173.5 U/dl Vs 80.5 U/dl, $P<0.001$, at 4 hr; 160 U/dl Vs 82.5 U/dl, $P<0.001$, at 8 hr; 191 U/dl Vs 100.5 U/dl, $P<0.001$, at 12 hr; 209 U/dl Vs 81.0 U/dl, $P<0.001$, at 24 hr].*
Conclusion. *HES may damp down the systemic inflammatory response and reduce endothelial cell dysfunction.*

Keywords: Hydroxyethyl starch; Endothelium; von Willebrand factor antigen; Microalbuminuria; Ischaemiareperfusion; Capillary permeability.

The biochemical and cellular events that mediate systemic inflammatory response cause activation of the endothelial cells in various vascular beds. Once activated these endothelial cells are able to participate in neutrophil–endothelial interactions.[1] This interaction is a needed defence mechanism, but may precipitate organ dysfunction if unchecked.[2] Cytokines such as interleukin-6 and interleukin-8, as well free radicals are some of the biochemical products involved in the inflammatory response. Acute phase proteins such as C-reactive protein, synthesised by the liver in response to the systemic inflammation, represent an index of the systemic inflammatory response.

Activated endothelial cells secrete von Willebrand factor (vWF) that serves a crucial role in mediating platelet adhesion to sites of endothelial damage.[3] Although it is secreted in small amounts constitutively, vWF is also released by stimulus-induced exocytosis of specialised secretory vesicles.[4] The appearance of large quantities of vWF in plasma can, therefore, be regarded as a surrogate marker of endothelial cell activation,[5] as the contribution from platelets is very small.[6]

Besides the cellular metabolic changes, activated endothelial cells also undergo ultrastructural changes. Morphologically, activated endothelial cells are more rounded with gaps appearing between adjacent endothelial cells.[7] The appearance of these gaps makes it easier for large molecules such as albumin to escape through the endothelial cell layer. This increase in albumin filtration by the endothelial layer can be quantified as an increase in the transcapillary escape of albumin[8] or as an increase in the urinary excretion of albumin—microalbuminuria.[9]

It has previously been suggested recently that hydroxyethyl starch (HES) can reduce the injurious effect of ischaemia and reperfusion (I–R).[10] In this randomised study we investigated the effect of HES, used as a plasma volume expander, on endothelial cell activation and systemic inflammation induced by I-R in humans.

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Patients and Methods

Approval for the study was obtained from the local research ethics committee. Forty patients scheduled for elective abdominal aortic aneurysm surgery were randomised to receive either hydroxyethyl starch (6% eloHAES, Fresenius-Kabi, Milton Keynes, UK) or gelatine solution (Gelofusine 4%, Braun, Sheffield, UK) as colloids. The 6% eloHAES solution consists of hexastarch molecules of average molecular weight 200,000 Da. The molecules are substituted at 1 and 6 and are slowly excreted via the kidneys after hydrolysis by amylase. The size and the degree of substitution give this molecule a different profile from other HES molecules.[11]

Patients with pre-existing microalbuminuria, defined as an overnight albumin excretion rates of 20–200 μg/min, and a creatinine of greater than 150 μmol/l were excluded from the study. Patients with occlusive disease were also excluded from the study. Randomisation was by sealed envelopes that were opened immediately prior to the start of surgery. All patients were anaesthetised using a standard technique of intravenous etomidate, fentanyl and vecuronium and anaesthesia was maintained with a mixture of nitrous oxide, oxygen and isoflurane with endotracheal intubation. Intra-operative and postoperative analgesia were achieved via an epidural catheter placed after induction of anaesthesia through which 2.5 mg of morphine in 5 ml of 0.35% bupivacaine was infused. Haemodynamic parameters were monitored by a trans-oesophageal Doppler probe, an indwelling triple lumen catheter in the internal jugular vein for central venous pressure (CVP) measurement, an indwelling radial artery line for continuous systemic blood pressure reading and for repeated arterial blood gas analysis. All patients received the same crystalloid. All patients received a litre of N-saline over 8 h before their operation. Immediately after induction of anaesthesia, patients were infused with 15 ml/kg of Hartman solution over 1 h followed by a maintenance fluid of 3 ml/kg of Hartman solution per hour during surgery. The study colloid was infused at induction of anaesthesia. The volume of colloid infused was based on various haemodynamic parameters so as to achieve a stable heart rate, CVP of 8–10 cm water, a steady mean arterial pressure and a urine output of greater than 40 ml/h. When the intra-operative haemoglobin fell below 10 g/dl, patients were transfused with red blood cells. Blood collected by the cell saver was infused in the normal way. Heparin was administered intravenously prior to aortic cross-clamp at a dose of 40 units/kg. Whether a tube graft or a bifurcated graft was used for the repair, only one leg was re-perfused at a time to prevent excessive haemodynamic changes. Blood and urine samples were taken once haemodynamic control was established.

Postoperatively, maintenance fluid replacement was crystalloid at 2 ml/kg/h. The study colloid and blood were infused as required to maintain a stable heart rate, CVP of 8–10 cm water, a steady mean arterial pressure and cardiac output. Blood was transfused so as to maintain a haemoglobin of 10 g/dl. The volume of crystalloid infused could be altered so as to maintain a urine output of greater than 40 ml/h postoperatively. Renal protection during surgery was achieved by infusion of 0.5 g/kg of mannitol given as an infusion of 10% solution prior to infra renal aortic cross clamp. Urine samples were collected at various time points for the determination of urinary albumin and creatinine as follows: pre-operatively (pre-op); before clamping of the abdominal aorta (Ind-Clp on); after clamp release (Clp off); 4-hourly thereafter for 12 h and daily for two post-operative days. At these same time points, blood samples were collected for measurement of plasma vWF.

Assessment of microalbuminuria

The urine samples were collected into sterile containers and kept at −70 °C until analysed. The analyses were performed on the thawed specimen in batches. The urine specimens were centrifuged to remove any sediment that might interfere with the assay. The microalbuminuria was determined by immunoturbidimetry using the Cobas Mira™ analyser. The results were expressed as a ratio of albumin to creatinine (ACR) in mg/mmol to allow for different urine flow rates.

Assay of vWF

Blood was collected into a vacutainer tube for von Willebrand factor antigen measurement. The samples were transported on ice prior to centrifugation. Platelet poor plasma was obtained by centrifugation of the blood sample at 3000*g*. The plasma was divided into aliquots and stored at −70 °C until batch analysed. The vWF concentrations were measured by an enzyme-linked immunosorbent assay (Dako, Copenhagen, Denmark). The coefficient of variation was less than 10%.

Table 1. Patients characteristics

	HES $n=20$	Gelofusine $n=20$
Age/year	71.2 (6.7)*	73.8 (9.5)*
Male:female	14:6	16:4
Duration of operation (min)	140 (80–240)†	135 (70–180)†
Clamp time (min)	54 (30–120)†	51 (22–105)†

* Mean (SD).
† Median (range).

Statistical analysis

The data were analysed using the Mann–Whitney test. The median was expressed with their quartiles. Intra group data were analysed using ANOVA. A P value of less than 0.05 was accepted as being statistically significant.

Results

Both groups of patients were equally matched. The ischaemia times were comparable (Table 1). The volumes of colloid and crystalloid were significantly different in the two groups of patients studied. Patients treated with HES infusion required less colloid [3000 ml (2500, 3437 ml) *vs* 3500 ml (3063, 4213 ml), $P<0.01$, Mann–Whitney test] and less crystalloid [4650 ml (4500, 4788 ml) *vs* 4975 ml (4762, 5137 ml), $P<0.01$, Mann–Whitney test] than the patients treated with gelofusine infusion. The volume of cell saved blood and banked blood transfused were not significantly different in the two groups of patients [960 ml (721, 1280 ml) *vs* 960 ml (680, 1280 ml), $P=$ns]. The haemoglobin on the first postoperative day was comparable in both groups of patients [10.6 g/dl (10.0, 12.0) *vs* 11.9 g/dl (10.7, 12.2), $P=$ns]. The platelet count was significantly lower on the first postoperative day in the HES treated patients than the gelofusine treated patients [121×10^3 (101×10^3, 141×10^3) *vs* 147×10^3 (128×10^3, 170×10^3), $P<0.05$, Mann–Whitney test]. The peak C-reactive protein was significantly lower in the patients treated with HES than those treated with gelofusine [142 mg/l (113, 196 mg/l) *vs* 246 mg/l (189, 291 mg/l), $P<0.01$, Mann–Whitney test].

The urinary excretion of albumin increased in both groups of patients during surgery indicative of an increase in capillary permeability. The excretion of albumin in the urine peaked following reperfusion of the extremities with release of the aortic cross clamp. Twenty-four hours after reperfusion, the urinary excretion of albumin returned to baseline levels. There was a differential increase in the urinary albumin: patients treated with gelofusine had a higher excretion of albumin in the urine. The differences were statistically significant immediately after release of the aortic cross clamp (23.3 *vs* 9.3 mg/mmol, $P<0.05$) and persisted for 4 h after reperfusion of the extremities (8.6 *vs* 1.9 mg/mmol, $P<0.01$). There was a secondary increase in the albumin excretion after 24 h in both groups of patients studied (Fig. 1).

The plasma vWF level decreased following release of the aortic cross clamp (Fig. 2). The plasma vWF levels at clamp release were comparable in both groups of patients (110 *vs* 96.5 U/dl, $P=$ns). Thereafter there was a significant increase in the plasma levels of vWF in the group of patients treated with gelofusine compared to the group of patients treated with HES [173.5 *vs* 80.5 U/dl, $P<0.001$, at 4 h; 160 *vs* 82.5 U/dl, $P<0.001$, at 8 h; 191 *vs* 100.5 U/dl, $P\leq0.001$, at 12 h;

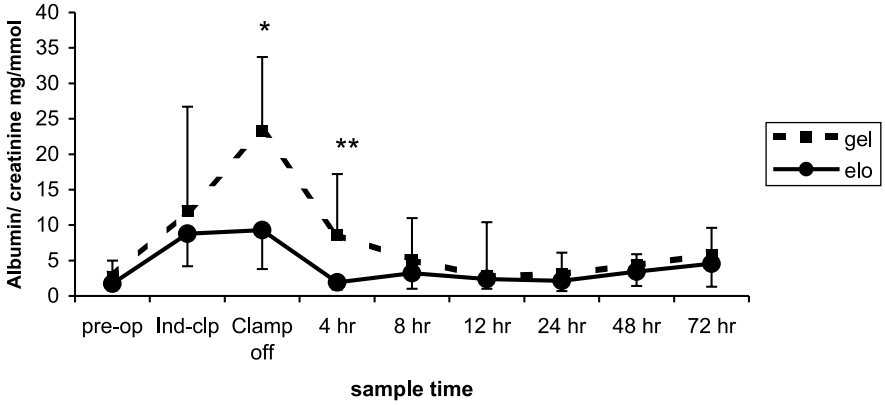

Fig. 1. Changes in ACR (median, interquartile range) in the perioperative period. *$P<0.05$, **$P<0.01$.

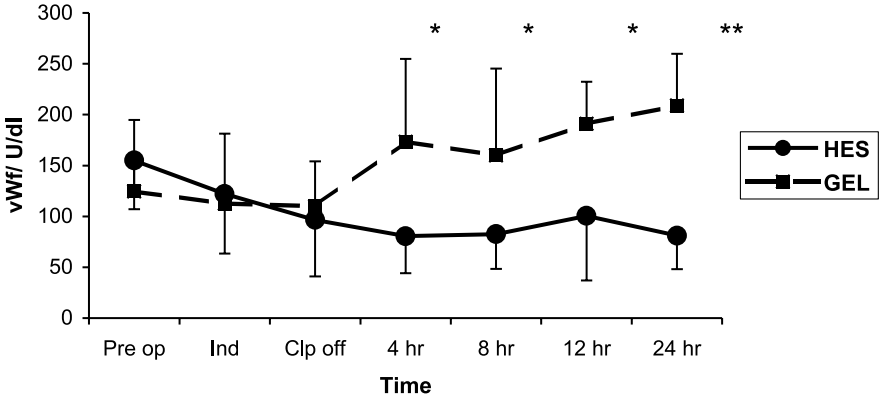

Fig. 2. Plasma vWF (median and interquartile range) in the perioperative period *$P<0.01$, **$P<0.001$.

209 *vs* 81.0 U/dl, $P<0.0001$, at 24 h]. During the first 24 h after reperfusion of the extremities, the plasma level of vWF in the HES treated remained relatively unchanged. On the first postoperative day, the plasma level of vWF remained significantly lower in the HES treated patients (259 *vs* 169 U/dl, $P<0.0001$).

Discussion

The early increase in the urinary excretion of albumin during surgery is consistent with previous observations that surgery induces an increase in the systemic capillary permeability.[9] The increase in capillary permeability is highest after release of the aortic cross clamp consistent with the reperfusion injury of the ischaemic extremities. There was a differential excretion of albumin in the urine in the two groups of patients studied suggesting that there was also a differential increase in capillary permeability (Fig. 1). This in turn is translated into the observed differential requirements of both colloid and crystalloid in these two groups of patients. The transient increase in microvascular permeability and its associated fluid shift has been shown to affect gas exchange.[12] It has previously been suggested that HES can exert an effect on the leaky capillaries by sealing the pores. Based on laboratory models it is suggested that HES molecules of molecular weight ranging from 100 to 300 kDa may be more effective at plugging the pores than HES molecules of larger molecular weights.[10] These experiments would suggest that the effect of HES on activated endothelial cells is purely biophysical. However, in trauma patients[13] as in this study the CRP levels in patients treated with HES were lower. CRP is an acute phase protein synthesised in response to pro-inflammatory mediators such as IL-6. The plasma IL-6 has previously been shown to be lower in patients treated with HES.[14] HES may, therefore, have anti-inflammatory properties.

Most of the plasma vWF is derived from endothelial cells rather than platelet suggesting that vWF is a good marker of endothelial dysfunction. Following reperfusion of the extremities, the plasma level of vWF decrease in both groups of patients. This decrease in the plasma level of vWF probably reflects an increase in the consumption of vWF by activated platelets rather than a decrease in their release.[15] The plasma vWF was comparable in both groups of patients after aortic clamp release consistent with a reperfusion injury of equal magnitude inflicted by free radicals. After the initial fall, the plasma vWF rose in the group of patients treated with gelofusine indicating an ongoing release of vWF by the activated endothelium. In contrast, the plasma vWF in the patients treated with HES did not change significantly after the initial fall following reperfusion. This would suggest that following the initial activation of the endothelium, HES might be exerting a stabilising effect on the endothelium, preventing further activation of endothelial beds. Evidence in support of this hypothesis is available from both *in vivo*[16] an *in vitro* study showing inhibition of endothelial cell activation by HES.[17] This inhibitory effect on the endothelium is translated into a lesser increase in capillary permeability and a lower CRP in the patients treated with HES.

vWF plays a vital role in platelet haemostasis. It mediates platelet adhesion to sites of vascular damage through interaction with platelet glycoprotein Ib. The platelet count in this study decreased transiently in the HES treated patients but remained above levels required for normal haemostasis. The importance of this decrease in the platelet count is not clear. Treatment with HES has been associated with an increased risk of bleeding through impairment of vWF. The larger the HES molecule, the higher is the risk of bleeding.[18] Impairment in its synthesis,

reduction in the release of vWF as well as accelerated elimination has been theorised.[19] Whether the persistent rise in plasma vWF is secondary to poor plasma clearance or sustained release from the activated endothelial cells could not fully be addressed in this study. As the plasma half-life of vWF is longer than the time intervals the blood samples were taken, the lower plasma concentration of vWF in the HES treated group could not satisfactorily be explained by the plasma clearance alone. Measurement of the propeptide, released in equimolar concentration but with a shorter half-life than the mature vWF,[20] might provide more information on the time scale of endothelial dysfunction.

The best way of assessing endothelial function is uncertain. The possible advantage of using endothelial cell function surrogates is that they reflect the net effects of a number of different molecular pathways. It appears that the effects of HES on the endothelium can be a double-edged sword that on one hand prevents excessive activation of the inflammatory process and on the other can interfere with haemostasis. This study shows that when used in the right pathophysiological states such as in abdominal aortic aneurysm repair, volume expansion with HES can confer significant benefits in terms of damping down the inflammatory cascade and endothelial cell dysfunction.

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